(54) Title: MONOCLONAL ANTIBODY TO AMYLOID PEPTIDE

(57) Abstract

Disclosed is a monoclonal antibody and specific-binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which monoclonal antibody and specific-binding fragment thereof does not react with other peptides of human origin. Also disclosed is a hybridoma cell line capable of producing the monoclonal antibody, a reagent composition which incorporates the monoclonal antibody or specific-binding fragments thereof and an immunoassay method for their use.

DESIGNATIONS OF "DE"

Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.

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MONOCLONAL ANTIBODY TO AMYLOID PEPTIDE

BACKGROUND OF THE INVENTION

Field Of The Invention

5   The present invention relates to neurological disorders, and, more particularly, to monoclonal antibodies that are specific for peptides associated therewith.

Brief Description Of The Prior Art

10   Alzheimer's disease is characterized by three major pathological markers. They are neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. Glenner, et al., Biochem. Biophys. Res.

15   Comm., 120:885 (1984) and Wisniewski, et al., Int. Symp. Dementia and Amyloid "Neuropathology", Suppl. 3:87 (1986). Alzheimer's cerebrovascular amyloid protein has been purified and a 24 amino acid residue sequence has been reported. Glenner, et al.,

20   Biochem. Biophys. Res. Comm., 122:1131 (1984). It has also been observed that the sequenced portion of the cerebrovascular amyloid peptide is part of the sequence of a precursor protein. Robakis, et al., Proc. Nat. Acad. Sci. USA, 84:4190 (1987) and

25   Robakis, et al., The Lancet, 1:384 (1987).
        Down's Syndrome is a disability characterized by the inheritance of an extra copy of chromosome 21 in each cell. Older persons afflicted with Down's Syndrome display dementia that resembles

30   Alzheimer's disease. The cerebral tissues of these individuals exhibit the same neuropathological findings of Alzheimer's disease, i.e., amyloid-containing neuritic plaques, neurofibrillary tangles and cerebrovascular amyloidosis. The amyloid

35   deposits of Down's Syndrome contain the same peptide

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as the amyloid deposits found in the brains of
Alzheimer's disease victims. See, Glenner et al.,
Biochem. Biophys. Res. Comm., 122:1131 (1984).

As applied to other peptides and antigens,
including many associated with various diseases,
monoclonal antibody products of hybridoma technology
have often proven valuable in studying factors
associated with these diseases and in diagnosis of
the disease in clinical settings. Kim, et al.,
Neuroscience Research Communications, 2:121 (1988)
discloses production and characterization of
monoclonal antibodies specific for the synthetic
cerebrovascular amyloid protein described in Glenner
et al., Biochem. Biophys. Res. Comm., 122:1131
(1984), supra.

SUMMARY OF THE INVENTION

Hybridoma technology is one of the most
important biological tools for the analysis of
complex antigens. Using the monoclonal antibodies of
the present invention, which are specific to recently
acquired amino acid sequences of beta - amyloid
precursor proteins, it has become possible to study
the beta-amyloid precursor protein which leads to
significant deposits of vascular and neuritic amyloid
plaques in Alzheimer's disease and Down's syndrome.
These monoclonal antibodies provide the potential for
non-invasive diagnosis thereof using body fluids such
as spinal fluid, serum or urine.

In one aspect, the invention provides a
monoclonal antibody and antigen - binding fragment
thereof which are specifically reactive with a
peptide whose concentration level is elevated in
individuals having Alzheimer's disease or Down's
syndrome as compared to individuals of substantially
the same age who are not so-afflicted and which do

not react with other peptides of human origin. The
monoclonal antibodies of the invention are primarily
of subclass IgG_1, while others are of a subclass
selected from IgG_{2a}, IgG_{2b} and IgM. Papain -
digestion produced antigen - binding fragments (e.g.
5   Fab) have also been shown to specifically recognize
amyloid plaque.

The monoclonal antibody is produced by a
hybridoma formed by fusion of cells from a myeloma
line, usually of mouse origin, and antibody-producing
10   cells, also usually of mouse origin, previously
immunized with a peptide whose concentration level is
elevated in individuals having Alzheimer's disease or
Down's syndrome as compared to individuals of
substantially the same age who are not so-afflicted
15   and which do not react with other peptides of human
origin, such as one which forms a portion of a
cerebrovascular amyloid plaque characteristic of
Alzheimer's disease or Down's syndrome. Preferably,
a mouse NSO myeloma line is used, particularly with
20   antibody-producing cells from a mouse previously
immunized with a peptide having a C-terminal sequence
comprising leu - val - phe - phe - ala - glu - asp -
val. Examples are monoclonal antibody SCVAP-2F9 and
monoclonal antibody SCVAP-4G8. Further in this
25   regard, the invention provides a hybridoma cell line
capable of producing the monoclonal antibodies
described above.

In another aspect, the invention provides a
composition for quantitatively determining a peptide
30   whose concentration level is characteristic of
Alzheimer's disease or Down's syndrome in an
individual. The composition comprises a monoclonal
antibody or antigen - binding fragment thereof in
accordance with the invention and a detectable moiety
35   which is directly or indirectly associated

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therewith. In one embodiment, the monoclonal
antibody is conjugated to an enzyme and the
detectable moiety comprises a chromogenic redox
substrate for the enzyme. In another embodiment, the
monoclonal antibody is conjugated to one partner of a
5   specific binding pair and the other partner of the
specific binding pair is conjugated to a detectable
moiety or a substance capable of rendering the moiety
detectable. One example of this embodiment is where
the monoclonal antibody or antigen - binding fragment
10   thereof additionally serves as an antigen of the
specific binding pair. The partner for the
monoclonal or fragment is an anti-immunoglobulin
antibody, usually anti - IgG and usually from a
species other than that of its partner, which is
15   labeled, such as with a fluorophore like fluorescein
isothiocyanate. Another example is where one partner
of the specific binding pair is selected from biotin
and its binding analogs and the other partner is
selected from avidin and its binding analogs.
20   Usually, the detectable moiety is a chromophore,
fluorophore or luminophore and the substance capable
of rendering it detectable is an energy donor or
catalyst therefor.

Further in this regard, the invention
25   provides an immunoassay method. The method comprises
contacting a sample, from the individual suspected of
having Alzheimer's disease or Down's syndrome, with
the composition of the invention and quantitatively
observing any detectable response.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph illustrating that the
relative OD reading between concentrations of SCVAP
in the range shown can be quantitated using the
35   method in accordance with the invention. The

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sensitivity of the method is in the range of 0.2 to
0.4 ng/100 ul of SCVAP in solution.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Example 1

Monoclonal Antibody (mAB) To Synthetic Cerebrovascular Amyloid Peptide (SCVAP) and Fragments

5   In the experiments reported by this example,
10 a synthetic polypeptide, an antigenic conjugate
thereof and tryptic fragments of the polypeptide were
used to prepare and characterize the monoclonal
antibodies of the invention.

15 ### Preparation of SCVAP and Fragments
          Preparation of SCVAP and its tryptic
fragments was as follows. A 24-residue synthetic
cerebrovascular amyloid peptide (SCVAP) corresponding
to the published sequence of the Alzheimer's
20 cerebrovascular amyloid peptide was obtained from
Peninsula Laboratories, Belmont, CA. The sequence
mentioned was published in Glenner, et al., Biochem.
Biophys. Res. Comm., 120:885 (1984). The preparation
as received was purified by reverse phase liquid
25 chromatography and the sequence was confirmed by
amino-terminal sequencing as described in Bobin, et
al., Acta Neuropathol., 74:313 (1987). The purified
peptide (1 mg) was conjugated to keyhole-limpet
hemocyanin (6 mg) with glutaraldehyde (3 ul) in
30 sodium phosphate (1.2 ml, 50 mM, pH 7.5) at 4°C for 2
hours to provide the SCVAP-KLH antigen.
          The purified peptide was also cleaved using
trypsin into fragments containing residues 1-5, 6-16,
6-24 and 17-24 as follows. The peptide (1 mg) in 0.1
35 M NH_4HCO_3 (500 ul) was treated with TPCK-treated

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trypsin (Cooper Biomedical, Malvern, PA) overnight at
37°C. The peptide fragments were resolved by
reverse-phase chromatography on a 4.6 x 50 mm C-8
column (J.T. Baker, Phillipsburg, NJ) eluted with a
linear gradient of 0-60% acetonitrile in 0.1%
5   trifluoroacetic acid for 40 minutes at 1 ml/minute.
The fragments were identified by amino analysis and
the sequences were determined to be as follows:

Fragment	Residues	Sequence
1	1-5	DAEFR
2	6-16	HDSGYQVHHQK
3	6-24	HDSGYQVHHQKLVFFAEDV
4	17-24	LVFFAEDV

Amino acids are designated by one-letter symbols in
15   accordance with the convention described, inter alia,
in Lehninger, Biochemistry (2nd Ed.), Worth
Publishers, Inc., New York, pg. 72 (1975). These
fragments were used, as described below, to
sublocalize the epitopes of the monoclonal antibodies
20   prepared as described herein.

Preparation of Hybrid Cell Lines
          Procedures for producing antibody-secreting
hybrid cell lines as described in Togashi, et al.,
25   Arch. Virol., 67:149 (1981) and modified as described
in Kim, et al., J. Clin. Microbiol., 18:331 (1983)
were applied as follows.
          BALB/CJ female mice (Jackson Laboratories,
Bar Harbor, ME) were immunized via the back foot pads
30   with Ribi adjuvant (200 ul) (Ribi Immunochem
Research, Inc., Hamilton, MT) containing SCVAP-KLH
antigen (25-50 ug). At 21 days after the initial
injection, these mice were immunized
intraperitoneally with SCVAP-KLH antigen (25-50 ug)
35   in Ribi adjuvant (200 ul). Four days prior to

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fusion, at 6 weeks after the last immunization, the
mice received intraperitoneal booster injections of
SCVAP (200 ug) without adjuvant.   Immune spleen cells
were harvested as described in Galfre, et al.,
Methods Enzymol., $\underline{3}$:73 (1981).   A mouse with the
highest titer after SCVAP-KLH antigen immunization
was selected for hybrid clone production.

NSO myeloma cells were obtained from Dr.
Choi, Kyung Hee of Albert Einstein Medical School,
New York, NY.   The immune spleen cells were incubated
with the NSO myeloma cells and fused at a ratio of
5:1 in the presence of polyethyleneglycol 1500
(Boehringer Mannheim, Indianapolis, IN) as the fusing
agent.   The cells were maintained in Iscoves modified
Dulbecco minimum essential medium containing 15%
fetal bovine serum, 10^{-4} M hypoxanthine, 1.6 x
10^{-5} M thymidine and 4.0 x 10^{-7} M aminopterin for
14 days.   Subsequent feedings with medium containing
only hypoxanthine and thymidine were performed on
days 18, 22 and 25.   After day 25, cells were fed
with medium that did not include hypoxanthine,
thymidine and aminopterin.   Normal BALB/CJ mouse
macrophages (6,000 cells per well in 96 wells of a
microtiter plate) as a feeder layer were used for the
initial hybridoma production.

Establishment of Hybrid Clones Secreting Anti-SCVAP
mABs

After the initial hybridoma production, as
described above, 5 x 10^{4} hybridoma cells in 1.0 ml
were added to each well of 96 well tissue culture
plates.   After 2 to 4 weeks of incubation, hybrid
cell growth was detected in 300 of 420 wells.   The
supernatant fluid in 122 of hybrids from the 300
wells were found to contain SCVAP-specific antibody
as determined by ELISA, as described below.

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Hybrids that produced SCVAP specific
antibody, as determined by the ELISA procedure, were
cloned three times by limiting dilutions to obtain
pure clones.   Normal BALB/CJ mouse macrophages (6,000
cells per well in 96 wells of a microtiter plate) as
5   a feeder layer were used here also. Fifty (50)
stable cloned hybrid cell lines were established and
are referred to here as purified antibodies. From
these, thirty (30) well-adapted, good antibody
producers were grown in tissue culture flasks and
10   further characterized.

ELISA Screening for Anti-SCVAP mABs

Hybridoma cells were tested for the
production of SCVAP antibodies by the ELISA technique
15   as described by Kim et al., J. Clin. Microbiol.,
18:331 (1983). SCVAP antigen was coated onto Falcon
96 well, 3911 Micro Test 111TM flexible assay plates
by adding 50 ul of SCVAP antigen (1 ul/ml) in
carbonate buffer (ph 9.6) and incubating overnight at
20   4°C. The substrate used was 3,3',5,5'-
tetramethylbenzidine (TMB) (1 ml of 10% stock
solution of TMB dissolved in dimethyl sulfoxide, 100
ml of 0.1 M sodium acetate/citric acid buffer, pH
6.0, 148 ul of 30% hydrogen peroxide). Substrate
25   solution was added to each well (100 ul/well) and
incubated at room temperature for 30 minutes. The
reaction was stopped by adding 2 M sulfuric acid (25
ul) to each well. The color change was measured with
a Dynatech Micro Elisa Auto Reader MR580, using a 450
30   nm filter.

The antibody titers as determined by ELISA
for tissue culture supernatant and ascites fluids
were mostly around 1:20,000 and 1:1,000,000,
respectively.

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Characterization of Immunoglobulin Subclasses

Monoclonal antibodies produced as described above were recovered from culture fluids and characterized by Ouchterlony double diffusion tests with goat anti-mouse antibody specific to IgG_1,

5   IgG_{2a}, IgG_{2b}, IgG_3 and IgM. The reagents were obtained from Meloy Laboratories, Inc., Springfield, VA. Most of the clones secreted the IgG_1 subclass of immunoglobulin; very few secreted IgG_{2a}, IgG_{2b} and IgM subclasses and none secreted the IgG_3

10   subclass.

Characterization of SCVAP mAB-Inducing Epitope

The SCVAP epitope for the monoclonal antibodies from the thirty (30) clones selected was

15   determined as follows. To identify the amino acid sequence(s) in SCVAP which are immunogenic epitopes to monoclonal antibodies produced against SCVAP, SCVAP was trypsin digested as described above and fragments containing residues 1-5, 6-24, 6-16 and

20   17-24 were obtained. When each of the monoclonal antibodies were tested against these peptide fragments by ELISA, all of them reacted with peptides containing the amino acid sequences 1-24, 6-24 and 17-24 and none reacted with those containing the 6-16

25   or 1-5 amino acid residues.

These data demonstrate that amino acid residues 17-24 are those which form the antigenic domain that induced production of the anti-SCVAP antibodies described above. It was surprising to

30   find that this sequence contained the preferred epitope. This region is composed of the sequence leu - val - phe - phe - ala - glu - asp - val, in which six of the eight residues are hydrophobic and are probably restricted in a beta-helix. According to

35   the rules that good epitopes should be hydrophilic

and mobile, one would not predict that this sequence
would be especially antigenic. Because these
antibodies react as strongly with purified
cerebrovascular amyloid protein and amyloid core
proteins as they do with SCVAP, it is concluded that
5   this epitope is equally exposed in the natural
amyloids and the synthetic peptide.

Example 2
Determination of Antigenic Determinant Commonality

10   In the experiments reported by this example,
monoclonal antibodies purified from mouse ascites
fluid were used in immunoassays in competition with
homologous and heterologous antibody preparations to
determine if they had epitopic sequences in common.

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Preparation of Purified mABs from Ascites Fluid

            From the cloned hybrid cell lines, prepared
as described in Example 1, that continued to produce
antibody for 3 months, ascites fluid was obtained by
20   intraperitoneal injection of 10^7 cells into BALB/CJ
mice which had been pretreated with 0.25 ml of
pristane (2,6,10,14-tetramethyl-pentadecane) 1 and 8
days before injection.

            Immunoglobulins of the IgG subclasses were
25   purified from the ascites fluid with the Affi-Gel
Protein A MAPS system (Bio-Rad Laboratories,
Richmond, CA). The purified IgG fractions from the
Protein A MAPS column were combined and dialyzed
against several changes of PBS and stored at -70°C
30   until used. Monoclonal antibodies obtained in this
manner are referred to herein as purified antibody.

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Anti-SCVAP mAB - Horseradish Peroxidase (HRP) Conjugate

HRP was conjugated to anti-SCVAP monoclonal antibodies from each of the purified preparations by the periodate method described in Wilson and Nakane, In Knapp et al(eds.), Immunofluorescence and Related Techniques, Elsevier Sci. Publ. Co., Amsterdam, pp. 215-225 (1978). The conjugate solution was stored at 70°C in small aliquots after adding 1% BSA, 50% glycerol and 0.01% Thimerosal.

Identification of Antigenic Determinants

For each monoclonal antibody a fixed amount of HRP-conjugated antibody (1:5,000 dilution of stock conjugate) was mixed with various amounts (1, 10, 100 ug/ml) of either unlabelled homologous or one of the other (heterologous) monoclonal antibodies and added to ELISA plate wells coated with limiting concentrations of SCVAP antigen.

All of the monoclonal antibodies reactive to residues 17-24 (Fragment 4) effectively competed with each other and, therefore, appear to be directed either against the same epitope, or overlapping epitopes present in the amino acid sequence of residues 17-24.

Example 3
Immunocytochemistry of Alzheimer-Specific mABs

In this example, culture supernatants of 44 initial clones which exceeded 1.5 OD units in ELISA on SCVAP-coated wells were screened for immunocytochemical staining of cerebral amyloid.

Immunostaining Procedure

All supernatants were diluted with equal volumes of PBS containing 20 mg/ml BSA and applied on

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6 um sections of paraffin-embedded, formalin fixed
Alzheimer cortex. Bound antibodies were visualized
using the avidin biotin complex technique (ABC Kit,
Vector Labs, Burlingame, CA) as described in Wang et
al., Acta Neuropathol.(Berl.), 62:268 (1984).
5   Selected supernatants were also tested on sections of
Alzheimer hippocampus, a non-demented aged subject
and tangles-containing neuronal cell bodies isolated
from Alzheimer cortex as described in Iqbal et al.,
Acta Neuropathol.(Berl.), 62:167 (1984). In some
10   instances tissue sections were dephosphorylated prior
to application of the antibodies using 43 ug/ml calf
intestinal alkaline phosphatase type VII (Sigma
Biochemicals, St. Louis, MO). In this regard, see
Grundke-Iqbal et al., Proc. Nat. Acad. Sci. USA,
15   83:4913 (1986) and Grundke-Iqbal et al., J. Biol.
Chem., 13:6084 (1986).

Immunostaining of Cerebral Amyloid - Results
                Fifty (50) percent of the culture
20   supernatants reactive with the 17-24 amino acid
sequence of SCVAP also strongly reacted with amyloid
present in neuritic plaques and cortical and
meningeal vessels both in brain sections of Alzheimer
patients and non-demented controls. No staining of
25   Alzheimer neurofibrillary tangles or
axons/neurofilaments either in tissue sections or in
isolated neurons was observed with any of the
supernatants.
                In the case of the tangles, access of the
30   monoclonal antibody tau-1 to microtubule associated
polypeptides , tau-1 is known to be blocked by
phosphorylation , as reported in Grundke-Iqbal,
P.N.A.S. USA, supra. Therefore, tissue sections were
also treated with alkaline phosphatase before
35   immunostaining with the anti-SCVAP antibodies.

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However, no change in immunostaining pattern or
intensity was observed when the tissue sections or
tangles containing neurons were dephosphorylated
prior to application of immunocytochemically reactive
or unreactive antibodies.

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Example 4
Immunohistology Using Alzheimer-Specific mABs

In the experiments reported by this example,
purified cerebrovascular amyloid peptides and
10   purified neuritic plaque amyloid core peptides were
used for testing of recognition by monoclonal
antibody specific for Fragment 4, described in
Example 1.

15   ### Preparation of Cerebrovascular Amyloid Peptides

Cerebrovascular amyloid material was
obtained from autopsy-derived meninges as described
by Glenner and Wong, Biochem. Biophys. Res. Commun.,
120:885 (1984). Cerebrovascular amyloid peptides
20   were isolated by size exclusion chromatography and
reverse-phase chromatography as follows.
Cerebrovascular amyloid was dissolved in 6 M
guanidine HCl-50 mM Tris HCl, pH 7.5. The solution
was dialyzed and lyophilized, and the residue of
25   amyloid peptides was dissolved in 70% formic acid,
0.1% acetonitrile. This preparation was
chromatographed on a 1 x 30 cm Pharmacia HR-12 size
exclusion column equilibrated with the preceding
solvent. The fractions containing cerebrovascular
30   amyloid peptide were dried and further fractionated
on a J.T. Baker C-8 column 0.4 x 10 cm in a solvent
containing 0-60% acetonitrile in 40% formic acid.

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Preparation of Neuritic Plaque Core Peptides

Neuritic plaque amyloid cores were prepared
as described by Bobin, et al., Acta. Neuropathol.,
74:313 (1987). Briefly, autopsy material shown to be
rich in neuritic plaque cores was homogenized in
5   phosphate-buffered saline (PBS), pH 7.0, and
centrifuged through 10 M sucrose to remove lipids.
The pellet was digested with pepsin and collagenase
and sedimented through Percoll. The layer containing
the amyloid cores was further washed with 6 M
10   guanidine HCl, 8 M urea, and 10% sodium deoxycholate
to remove adherent lipids and cerebrovascular
amyloid. Neuritic plaque amyloid core peptides were
purified by the same procedure as used for the
purification of the cerebrovascular amyloid peptides,
15   except that the cores were initially dissolved in 90%
formic acid.

Anti-SCVAP Fragment 4 mAB to Amyloid Core and Peptide

Monoclonal antibodies specific for amyloid
20   residues (Fragment 4) selectively stained both plaque
core amyloid and cerebrovascular amyloid in brain
tissue from patients with histologically confirmed
Alzheimer's disease. These monoclonal antibodies
were further tested for their immunoreactivity by
25   ELISA using 96 well plastic plates coated with the
purified neuritic plaque core amyloid peptides and
cerebrovascular amyloid peptides prepared as
described above. All of these monoclonal antibodies
reacted well with peptides from both amyloid core and
30   cerebrovascular plaque by ELISA.

Example 5

Quantitative Diagnostic Alzheimer ELISA Method

A quantitative ELISA procedure for the
35   detection of SCVAP in solution using the monoclonal

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antibodies of the present invention was developed as
follows.

Fifty (50) ul of various dilutions of
monoclonal antibody SCVAP 4G8-HRP conjugate (a
specific monoclonal antibody reactive to residues
17-24 of Alzheimer amyloid peptide conjugated to
horseradish peroxidase) were titrated by ELISA on
wells coated with the maximum amount of SCVAP antigen
(5 ug/ml).

The dilution of monoclonal antibody SCVAP
4G8-HRP conjugate that gave a relative OD reading
close to 1.0 when incubated with TMB for 30 minutes
as described above was determined. This
concentration of monoclonal antibody SCVAP 4G8-HRP
conjugate was mixed with an equal volume of SCVAP
solution containing varying concentrations of SCVAP
and incubated overnight at 4°C. The amount of
unadsorbed monoclonal antibody SCVAP 4G8-HRP
conjugate was then measured by titrating the mixture
on ELISA 96 well plates coated with 5 ug/ml of
SCVAP. After 4 hours of incubation at room
temperature the ELISA OD was determined by using TMB
as the substrate.

As shown in Fig. 1, the relative OD reading
between concentrations of SCVAP in this range can be
quantitated using this method. The sensitivity of
the method is in the range of 0.2 to 0.4 ng/100 ul of
SCVAP in solution.

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WHAT IS CLAIMED IS:

1. A monoclonal antibody or antigen - binding fragment thereof which is specifically reactive with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin.

2. The monoclonal antibody or binding fragment of claim 1 which is specifically reactive with a cerebrovascular amyloid protein whose elevated levels are characteristic of Down's syndrome and Alzheimer's disease.

3. The monoclonal antibody or binding fragment of claim 1 which is of subclass IgG_1.

4. The monoclonal antibody or binding fragment of claim 1 which is of a subclass selected from the group consisting of IgG_{2a}, IgG_{2b} and IgM.

5. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and antibody-producing cells from a mouse previously immunized with a peptide whose concentration level is elevated in individuals having Down's syndrome or Alzheimer's disease as compared to individuals of substantially the same age who are not so-afflicted and which does not react with other peptides of human origin.

6. The monoclonal antibody or binding fragment of claim 1 which is produced by a hybridoma formed by fusion of cells from a mouse myeloma line and

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antibody-producing cells from a mouse previously
immunized with a cerebrovascular amyloid protein
whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome.

5   7.   The monoclonal antibody or binding fragment of
claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse NSO myeloma line and
antibody-producing cells from a mouse previously
immunized with a peptide whose concentration level is
10   elevated in individuals having Down's syndrome or
Alzheimer's disease as compared to individuals of
substantially the same age who are not so-afflicted.

8.   The monoclonal antibody or binding fragment of
15   claim 1 which is produced by a hybridoma formed by
fusion of cells from a mouse NSO myeloma line and
antibody-producing cells from a mouse previously
immunized with a cerebrovascular amyloid protein
whose elevated levels are characteristic of
20   Alzheimer's disease or Down's syndrome.

9.   A monoclonal antibody which is produced by a
hybridoma formed by fusion of cells from a mouse NSO
myeloma line and antibody-producing cells from a
25   mouse previously immunized with a peptide having a
C-terminal sequence comprising leu - val - phe - phe
- ala - glu - asp - val, or an antigen - binding
fragment of said monoclonal antibody.

30   10. A monoclonal antibody selected from the group
consisting of monoclonal antibody SCVAP 2F9 and
monoclonal antibody SCVAP 4G8, or an antigen -
binding fragment of said monoclonal antibody.

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11. A hybridoma cell line capable of producing the
monoclonal antibody of claim 1.

12. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a myeloma line and
antibody-producing cells previously immunized with a
peptide whose concentration level is elevated in
individuals having Down's syndrome or Alzheimer's
disease as compared to individuals of substantially
the same age who are not so-afflicted.

13. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse myeloma line
and antibody-producing cells from a mouse previously
immunized with a peptide whose concentration level is
elevated in individuals having Down's syndrome or
Alzheimer's disease as compared to individuals of
substantially the same age who are not so-afflicted.

14. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse NSO myeloma
line and antibody-producing cells from a mouse
previously immunized with a peptide whose
concentration level is elevated in individuals having
Down's syndrome or Alzheimer's disease as compared to
individuals of substantially the same age who are not
so-afflicted.

15. The hybridoma cell line of claim 11 which is
formed by fusion of cells from a mouse NSO myeloma
line and antibody-producing cells from a mouse
previously immunized with a peptide having a
C-terminal sequence comprising leu – val – phe – phe
– ala – glu – asp – val.

16. A composition for quantitatively determining a peptide whose elevated levels are characteristic of Alzheimer's disease or Down's syndrome in an individual, which composition comprises the monoclonal antibody or specific - binding fragment of claim 1 and a detectable moiety which is directly or indirectly associated therewith.

17. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is conjugated to an enzyme and said detectable moiety comprises a chromogenic redox substrate for said enzyme.

18. The composition of claim 16 wherein said monoclonal antibody or specific - binding fragment is specifically bindable with a substance attached to said detectable moiety.

19. The composition of claim 18 wherein the substance attached to said detectable moiety is an antibody specifically bindable with an immunoglobulin.

20. The composition of claim 16 wherein said monoclonal antibody is conjugated to one partner of a specific binding pair and which further comprises the other partner of said specific binding pair conjugated to said detectable moiety or a substance capable of rendering said moiety detectable.

21. The composition of claim 20 wherein one partner of said specific binding pair is selected from the group of biotin and its binding analogs and the other partner of said binding pair is selected from the group of avidin and its binding analogs.

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22. The composition of either of claims 20 or 21
wherein said detectable moiety is a chromophore,
fluorophore or luminophore and said substance capable
of rendering said moiety detectable is an energy
donor or catalyst therefor.

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23. A method for quantitatively determining a peptide
whose elevated levels are characteristic of
Alzheimer's disease or Down's syndrome in an
individual, which method comprises contacting a
10   sample from said individual with the composition of
any of claims 16-20 and quantitatively observing any
detectable response.

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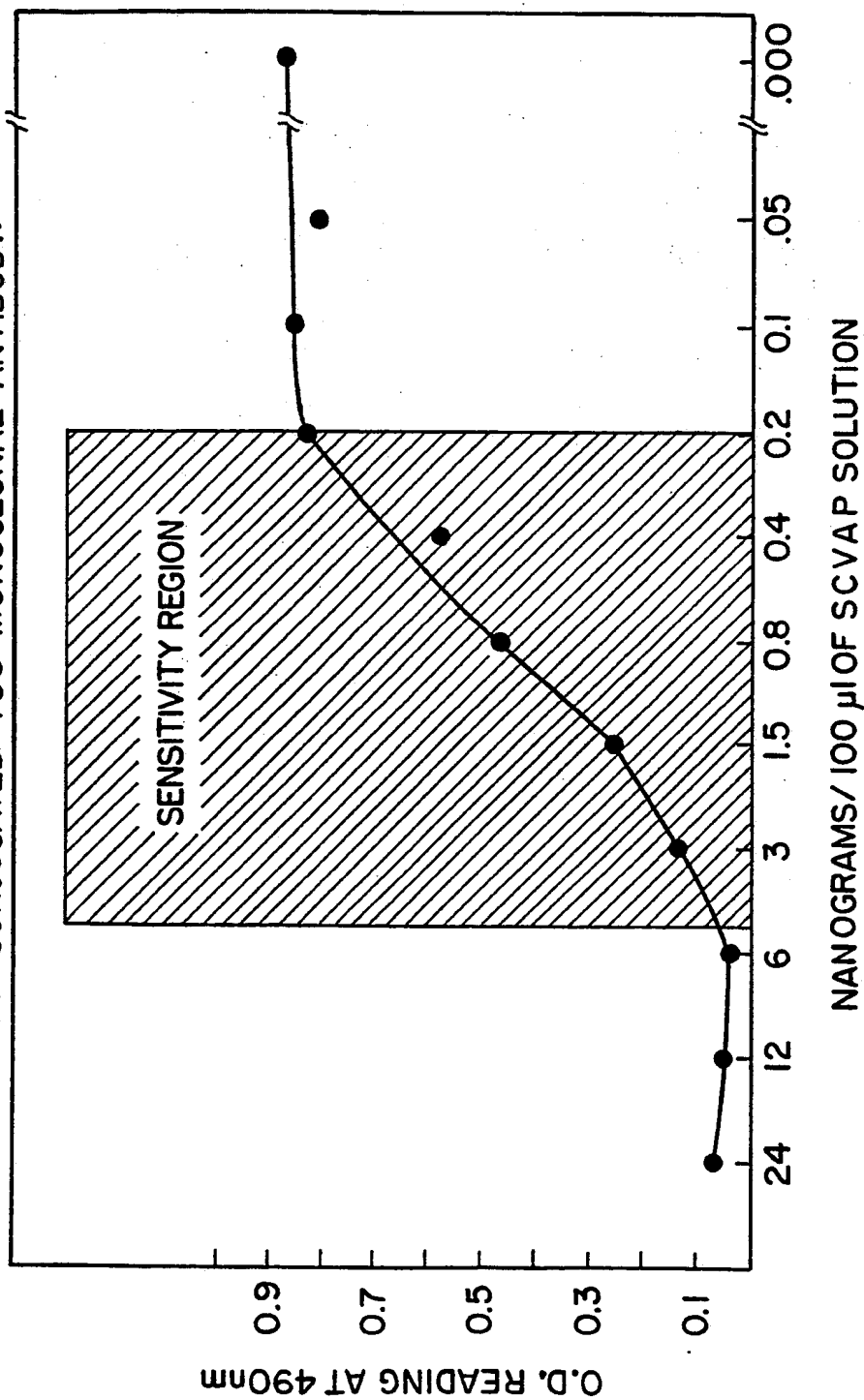QUANTITATION OF SCVAP BY A TWO STEP INCUBATION ELISA METHOD USING HRP–CONJUGATED 4G8 MONOCLONAL ANTIBODY.

FIG.1

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 3

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12N 5/20; C07K 15/28; G01N 33/533,534,535

U.S. CL: 530/387,388; 435/240.27, 188, 7.

II. FIELDS SEARCHED

Minimum Documentation Searched 4

Classification System	Classification Symbols
U.S.	530/387,388, 435/7, 188, 240.27; 935/104,108, 110

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 6

Databases: STN Online (File CA, File Biosis); ALPS; For: monoclonal antibody, amylord, cerebrovascular, Alzheimers, protein, polypeptide.

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

Category *	Citation of Document, 16 with indication, where appropriate, of the relevant passages 17	Relevant to Claim No. 18
X Y	US, A, 4,666,829 (GLENNER ET AL.) 19 May 1987 see the entire document.	1-8 9-23
Y	Robakis et al., "Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides", _Proc. Natl. Acad. Sci_ 84; 4190-4194, Published June 1987. see especially p. 4192, Fig 2.	1-23
Y	Selkoe et al., "Conservation of brain amyloid proteins and humans with Alzheimer's disease" _science_ 235: 873-877, Published 20 February 1987. see especially page 874, column 1.	1-23
Y	Glenner et al, "Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein" _Biochem. Biophys. Res. Comm._ 120: No. 3, 885-890. Published 16 May 1984. see especially page 889.	1-23
Y	Glenner et al, "Alzheimer's disease and Down's Syndrome: sharing of a unique cerebrovascular amyloid fibril protein" _Biochem. Biophys. Res. Comm._ Vol. 122 No. 3: 1131-1135. Published 16 August 1984. see especially pages 1131 and 1133, Table 1.	1-23

* Special categories of cited documents: 15

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 2	Date of Mailing of this International Search Report 2
25 JUNE 1990	2 5 JUL 1990

International Searching Authority 1	Signature of Authorized Officer 20
ISA/US	PAULA HUTZELL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Kang et al., "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surfacen receptor" _Nature_ 325: 733-736 published 19 February 1989. see especially page 735.	1-23
Y	Wong et al., "Neuritio plaques and cerebrovascular amyloid in Alzheimer disease are antigenically related" _Proc. Natl. Acad. Sci. USA_ 82: pages 8729-8732. Published December 1985. see especially page 8729.	1-23
Y	Goding, "Monoclonal antibodies: Principles and Practice" Published 1983 by Academic Press, INC. (LONDON), see pages 56-97 and pages 209-249, especially pages 230-235.	1-23

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers ____ . because they relate to subject matter [1] not required to be searched by this Authority, namely:

2.☐ Claim numbers ...... , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out [1], specifically:

3.☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet (2) (Rev. 4-90)

III. DOCUMENTS CONSIDERED TO BE RELEVANT   (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No [18]
X — Y	Scicutella et al., "Alz-so appears to be able to distinguish between the neuritic plaques of Alzheimer's disease and those of normal aging" soc. Neurose;Abstr. 13:1150, Published 1987. see abstract 316.5	1-8, 23 — 9-22
X — Y	Wolozin et al., "Re-Expression of a developmentally regulated antigen in Alzheimers Disease and Down's Syndrome" Soc. Neurosci; Abstr. 13:1150 Published 1987. See Abstract 316.6	1-8, 23 — 9-22